Review

Immune reconstitution in HIV-1 infected subjects treated with potent antiretroviral therapy

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The introduction of potent antiretroviral drug regimens contributed to a decline in HIV-1 associated morbidity and mortality. Clinical observations of spontaneous remission of previously untreatable opportunistic infections in subjects on highly active antiretroviral therapy (HAART) reflect the substantial degree of immune reconstitution which can be achieved by those therapies. A biphasic increase of CD4+ T lymphocytes has been reported including naive (CD45RA+) and memory (CD45RO+) cell subsets. Proliferative lymphocyte responses to recall antigens and mitogens are enhanced over time, while T lymphocyte activation is largely reduced and T cell receptor (TCR) repertoires are partly restored. Proliferative lymphocyte responses specific to HIV-1 antigens, in contrast, remain weak. A complete normalisation of HIV-1 associated immunological alterations has not been reported so far, but the observation period of subjects on potent antiretroviral therapies is still relatively short.
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Introduction

HIV-1 infection is associated with a diverse range of immunological alterations, which results in a progressive immunodeficiency rendering the infected individual susceptible to opportunistic infections and malignancies. The combination of nucleoside analogues and a protease inhibitor or a non-nucleoside reverse transcriptase inhibitor, which is also known as highly active antiretroviral therapy (HAART), is able to suppress very efficiently viral replication and is now regarded as the standard of care.[1] Although the ultimate goal of antiretroviral therapy is the cure of HIV infection and the eradication of the virus, recent reports suggest that the achievement of those aims will be difficult with currently available therapeutic options.[2–4] In particular, HIV-1 may persist in cellular viral reservoirs including latently infected CD4+ T lymphocytes, macrophages, and follicular dendritic cells. Anatomical reservoirs such as the central nervous system may be poorly accessible for certain drugs, which ultimately may limit the therapeutic antiretroviral efficacy in those compartments.

Despite those problems, HAART leads to a substantial quantitative and qualitative reversion of HIV-1 associated immunodeficiency, which has also been reflected by the dramatic decline of HIV-1 associated morbidity and mortality in the past 2–3 years.[5]

Effect of HAART on T lymphocyte subsets

One of the hallmarks of HIV infection is the progressive loss of CD4+ T lymphocytes. HAART leads to a significant increase of CD4+ T lymphocytes, even in subjects with highly advanced stages of the disease,[6–8] which approaches the normal range in a proportion of patients within 2 years.[9 10] Even if not all individuals reach normal numbers of CD4+ T lymphocytes after that time, a partial immune restoration may already sufficiently protect those subjects against opportunistic infections.[11] More detailed information on the recovery of CD4+ T lymphocytes resulted from the analysis of phenotypic subsets of T lymphocytes.[12 13] CD4+ T lymphocytes express either CD45RA+, which defines the subset of antigen naive cells or CD45RO+, which characterises the memory cell subset. Naive cells originate typically from the thymus and remain in a resting state until they encounter foreign antigen. When antigen and co-stimulatory signals are presented, naive cells become activated and switch to the memory phenotype. Cells bearing the memory phenotype have effector function and have the potential to respond faster to antigen. In early HIV-1 infection, memory (CD45RO+) CD4+ T cells are preferentially depleted while in advanced stages of the disease the proportion of naive cells (CD45RA+) declines.[14–16]

Within months of initiation of HAART, a biphasic increase of CD4+ T lymphocytes has been observed.[12 13 17 18] This initial change of CD4+ T cells is mainly the result of an increase of memory cells and is followed 2–3 months later by a smaller second phase increase of CD4+ T lymphocytes consisting of both naive and memory cells (fig 1).

It has been suggested that a rapid redistribution of CD4+ T lymphocytes trapped in lymphoid tissue, rather than the proliferation of new cells may be the main mechanism responsible for the initial rapid rise of CD4+ T cells.[17] In fact, it has recently been demonstrated that CD4+ T cell turnover does not change in the first months after the initiation of HAART, which supports the concept of a redistribution of cells.[19 20] Trapped T lymphocytes may be released from lymphoid tissue when cytokine and chemokine levels begin to decline in inflamed lymphoid tissue.[21] The second phase increase of CD4+ T cells may indeed be the

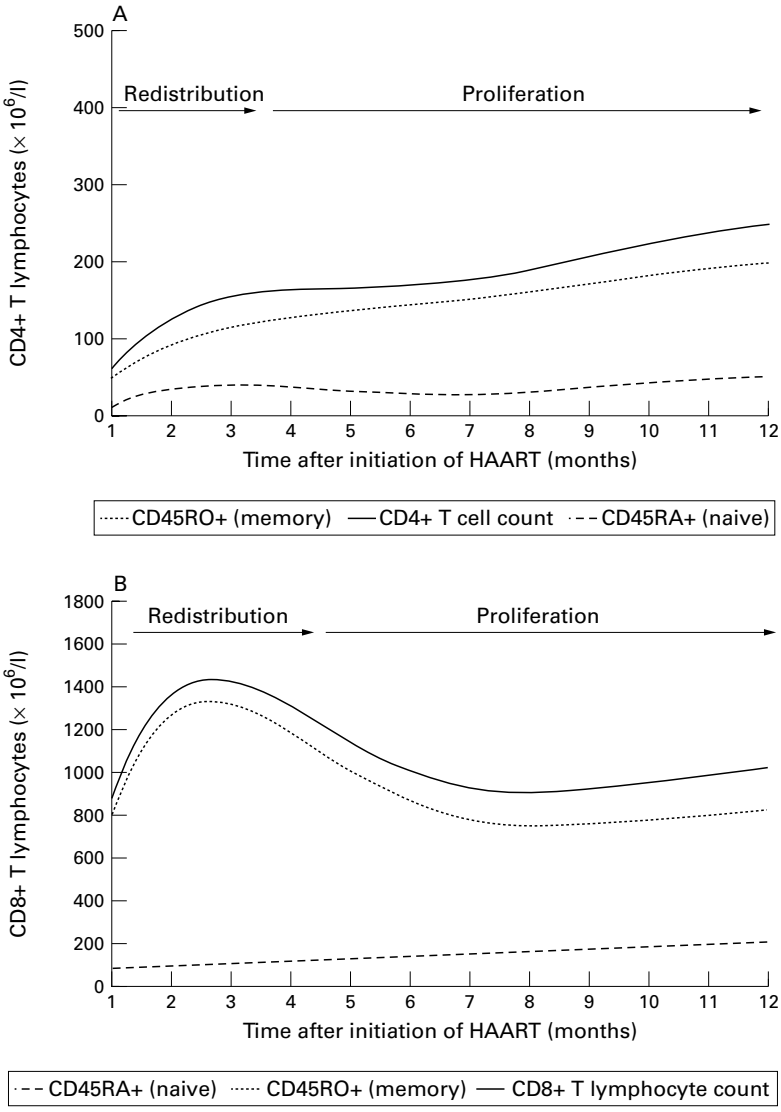

Figure 1 Time course of naive and memory T lymphocytes in a representative subject on HAART. (A) CD4+ T lymphocytes. (B) CD8+ T lymphocytes. It has been suggested that the initial rapid increase of lymphocytes is the result of a redistribution of lymphocytes trapped in the lymphoid tissue, which are released into peripheral circulation upon initiation of an effective antiretroviral therapy. The subsequent slow increase of cells may be the result of the regeneration of new lymphocytes.

result of newly regenerated cells. During this time the proportion of CD4+ T lymphocytes expressing Ki67, a marker of cell proliferation, increases.[19]

Recent reports indicate that virological and immunological responses may not always be concordant.[22 23] In one study, 11% of subjects had a significant increase of CD4+ T lymphocytes despite persistently high HIV RNA levels, which were accompanied by a decline of AIDS defining events.[22] In contrast, another 11% of individuals demonstrated no significant increase of CD4+ T lymphocytes despite undetectable plasma HIV RNA. AIDS defining events were frequent in this group reflecting the persistent immunodeficiency. This observation suggests that the degree of immune restoration relies not only on the suppression of viral replication and HIV RNA levels, but may also depend on the cytopathic properties and the fitness of the viral strain as well as the underlying condition of the immune system.[23 24]

In untreated HIV-1 infection, the number of CD8+ T lymphocytes usually remains elevated over years at a fairly constant level and then dramatically declines towards the final stages of the disease. CD8+ T lymphocytes show a similar biphasic course on HAART as CD4+ T lymphocytes.[17] Memory CD8+ T lymphocytes expand rapidly within weeks resulting in an overall increase of total CD8+ T cell counts, but decline again in subsequent months.[12 13 17] The proportion of naive CD8+ T lymphocytes increases slowly keeping the level of total CD8+ T lymphocytes at an almost constant level.[10]

An extensive immune activation of T lymphocytes is one of the cardinal features of HIV infection and may lead to anergy and increased apoptosis of those cells. The proportion of activated T lymphocytes increases towards later stages of the disease and is correlated with levels of plasma HIV RNA.[25] HAART reduces rapidly the number of activated CD4+ and CD8+ T lymphocytes as measured by the expression of CD38 and HLA-DR antigens.[12 13 26]

How new T lymphocytes are generated

The state of the immune system and in particular, the number of naive T cells before therapy may determine the maximum level of immune restoration which can be achieved.[13] However, there is some uncertainty as to how new T lymphocytes are generated in HIV-1 infected subjects on HAART.

In mice, thymic dependent and thymic independent pathways of T cell regeneration have been reported. The thymus is involved in primary T cell development including the selection of appropriate cell clones, which are able to recognise foreign antigen, but are tolerant to self antigen. Extrathymic differentiation of bone marrow progenitors has been detected in gut, liver, and bone marrow.[27–29] However, the contribution of extrathymic differentiation of T cells to the peripheral T cell pool appears to be minimal.

Alternatively, a dramatic expansion of preexisting mature T cells has been observed in athymic mice after T lymphocyte depletion.[30–32] This renewal of T cells has been termed peripheral expansion, which appears to be mainly antigen driven.[33]

In humans, the thymus progressively involutes from birth to adulthood. HIV infected children on HAART, who are younger than 3 years old with a presumably active thymus, consequently showed a more rapid restoration of naive CD4+ T lymphocytes than older children or adults.[34] Although it has recently been shown that the thymus may keep some residual activity in adults,[35] it is likely that peripheral expansion of mature T lymphocytes is the main pathway responsible for the restoration of T cells in adults.[36] This mechanism may have some limitations. The thymus generates a diversity of T cells with different T cell receptors (TCR), which are capable of recognising a huge spectrum of foreign antigen. HIV infection leads to a severe disruption of this repertoire,[37] which may result in a poor recognition of certain antigens or even the lack of the antigen specific immune response. Peripheral

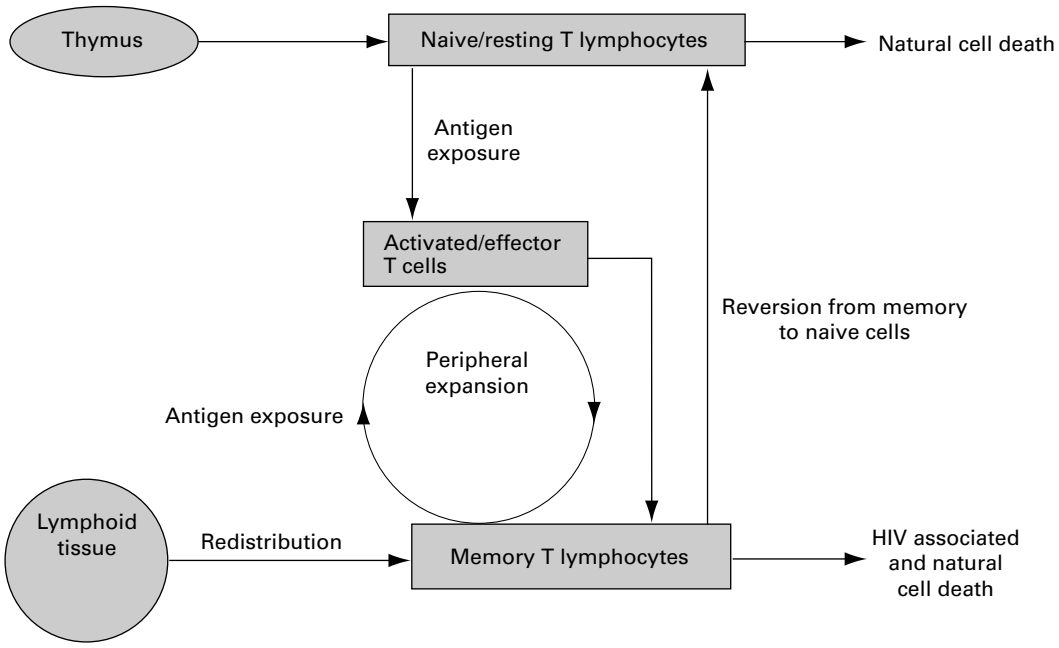

Figure 2 Possible pathways involved in the restoration of T lymphocytes. The thymus has an important role in very young children, but loses its significance in adults, which leaves peripheral expansion of mature T lymphocytes as the major mechanism for the restoration of lymphocytes. The reversion of memory cells to naive cells has been demonstrated in mice, but no conclusive data are available for humans, which would provide enough evidence that this mechanism is significantly involved in the immune restoration process in HIV-1 infected patients on HAART.

expansion of mature T cells multiplies only pre-existing T cell clones. This mechanism is therefore unlikely to completely correct perturbations of the TCR repertoire. Most studies with a relatively short observation period of 3–12 months reported an incomplete restoration of TCR repertoires of CD4+ and CD8+ T lymphocytes in HIV-1 infected adults and provide some evidence for this hypothesis.[37–39] The long term follow up of those subjects will show whether the T cell repertoire can be completely restored. Proposed pathways of immune reconstitution are shown in figure 2.

Effect of HAART on T lymphocyte function

Functional defects commonly precede the decline of CD4+ T cell counts including poor proliferative lymphocyte responses to mitogens and recall antigens as well as reduced cytokine production in response to antigens.[40–43] It is therefore important not just to rely on the restoration of lymphocyte numbers, but to assess lymphocyte function, which may provide additional information on the degree of immune competence.

Several studies have demonstrated that HAART increases proliferative lymphocyte responses to recall antigens such as tetanus toxoid, streptokinase/streptodornase or mitogens such as phytohaemagglutinin and pokeweed antigen.[12 38 44–46] Normal levels were not attained in studies with an observation period of 1–1.5 years, which indicates that proliferative T lymphocyte responses improve slowly.[46–49]

The restoration of an efficient immune response against opportunistic pathogens is of great clinical importance, in particular with regard to the discontinuation of primary and secondary antimicrobial prophylaxis. HAART significantly enhances proliferative responses to CMV, candida, *Mycobacterium avium*, and tuberculin.[13 38 50 51] In a recent study evaluating the CMV specific immune response, evidence has been provided that HAART is also able to increase the number of CD4+ T lymphocytes with pathogen specific effector function, as measured by the production of intracellular effector cytokines using a new flow cytometric technique.[52]

In two investigations factors have been studied which may predict the recovery of functional properties of CD4+ T lymphocytes in individuals treated with HAART. Those subjects with a significant improvement of proliferative lymphocyte responses had a more sustained viral load suppression, a greater CD4+ T cell increase, and an early increase of memory and naive CD4+ T lymphocytes.[51 53]

A crucial factor in the pathogenesis of HIV-1 infection is the lack of an efficient immune response to HIV-1 that would ultimately eradicate the virus. The mechanism by which the virus is able to escape the HIV-1 specific immune response remains speculative. The restoration of the HIV-1 specific immune response is therefore of particular interest. Several short term studies over 3 and 6 months reported a moderate enhancement of proliferative lymphocyte responses to HIV-1 antigens,[46 54] while other studies reported a lack or only a transient increase of the proliferative lymphocyte response despite a significant response to non-HIV specific antigens such as CMV.[13 50 55]

The reason for this poor HIV-1 specific reactivity is unclear. Proposed explanations include the selective depletion of HIV-1 specific T cell clones during HIV-1 infection, HIV-1 specific anergy, or an impaired antigen presentation. The last hypothesis raises the question of whether the lack of an efficient immune response to HIV-1 is due to a defect in

the network of follicular dendritic cells which serve as antigen repository and interact with B and T lymphocytes. This network of cells is progressively destroyed during the natural course of HIV-1 infection.[56] A recent investigation using immunohistochemical examinations of tonsils and lymph nodes provides evidence that HIV-1 specific pathological changes of the follicular dendritic network can be slowly reversed by HAART.[57]

In contrast with improvements of proliferative responses, the number of HIV-1 specific cytotoxic CD8+ T lymphocytes (CTL) drops exponentially 1–2 weeks after initiation of HAART and continues to decline after plasma HIV RNA has reached undetectable levels.[58 59] Similarly, a decline of HIV-1 specific CD4+ T lymphocytes has been observed in individuals on HAART.[60] Those findings suggest that additional vaccination strategies may be required to maintain the HIV-1 specific cytotoxic immune response in subjects on HAART.

A further area of interest is the humoral immune response to HIV-1. In one study, untreated HIV-1 infected patients with CD4+ T cell counts $<200 \times 10^6$/l, who were vaccinated against influenza, failed to induce a significant antibody response while subjects on HAART developed substantial antibody titres.[61]

HIV-1 infection causes severe perturbations of the cytokine network including an increase of proinflammatory cytokines such as tumour necrosis factor α (TNF-α) or interleukin 6 (IL-6) and a reduction of T helper 1 (Th1) type cytokines such as IL-2. Observations of an improved IL-2 and IL-12 secretion as well as a decline of TNF-α in individuals on HAART indicate that cytokine perturbations may be corrected by HAART.[44 49 62 63] Recent reports also suggest that HAART positively influences the secretion of chemokines resulting in increased RANTES levels which may help to suppress viral replication.[46 64] The effect of HAART on the immune system is summarised in table 1.

Immune restoration in early HIV-1 infection

The protracted course and the uncertainty of a full immune reconstitution have brought into question whether an early antiretroviral therapy during primary HIV-1 infection may prevent the subsequent severe and possibly irreversible HIV associated immunodeficiency.

In one series, 16 subjects with primary HIV-1 infection were treated with zidovudine, lamivudine, and indinavir over 1 year. The early initiation of therapy resulted in a sustained suppression of plasma HIV RNA and a continuing rise in circulating CD4+ T lymphocytes.[65] Moreover, the number of CD4+ T lymphocytes including the naive and memory subset reached normal values at 52 weeks. No significant perturbations of the TCR repertoire were detected. Naive CD4+ T lymphocytes recovered more rapidly in subjects who received therapy before the appearance of HIV-1 antibodies than individuals with a complete antibody response before therapy. Those findings suggest that the time between the onset of HIV-1 infection until the commencement of therapy may play a crucial role in determining the rate of the recovery of CD4+ T lymphocytes. In contrast, the number of CD8+ T lymphocytes did not change significantly over time. Memory CD8+ T lymphocytes dropped rapidly and were only slightly elevated at 1 year, while the naive subset of CD8+ T lymphocytes was maintained at a high level. CD8+ T lymphocyte activation as measured by the expression of surface antigens CD38 and HLA-DR was correlated with the plasma HIV RNA levels and decreased significantly during the observation period.

Table 1 Impact of HAART on the immune system

Increase of CD4+ T lymphocytes including memory and naive cells
Partial restoration of T cell receptor repertoires
Reduced lymphocyte activation
Improved proliferative lymphocyte responses to mitogens
Improved proliferative lymphocyte responses to recall antigens
Modest improvement of proliferative lymphocyte response to HIV-1 specific antigens
Increased number of pathogen specific CD4+ T lymphocytes
Improved cytokine/chemokine secretion
Improved humoral immune response
Improvement of the network of follicular dendritic cells

Limited data are available on the recovery of functional properties of T lymphocytes during primary HIV-1 infection. In 11 acutely infected subjects receiving HAART a restoration of lymphoproliferative responses to candida and tetanus toxoid comparable with levels observed in immunocompetent subjects has been observed after 3 months of therapy.[66] Moreover, a sixfold increase in the lymphoproliferative response to HIV-1 p24 antigen has been noted. Similarly, a vigorous proliferative response of CD4+ T lymphocytes to HIV-1 p24 antigen has been reported in three acutely HIV-1 infected subjects treated with HAART before HIV-1 seroconversion.[67] These results are in contrast with the poor proliferative HIV-1 specific responses in individuals treated in advanced stages of HIV-1 infection and suggest that the immune restoration may be more efficient if antiretroviral therapy is initiated early.

The preliminary data in a small number of subjects with primary HIV-1 infection certainly need further confirmation. A drawback of a therapeutic intervention during primary HIV-1 infection is the potential risk of an early development of drug resistance. Furthermore, the use of HAART is frequently associated with adverse events. A therapy initiated during primary HIV-1 infection therefore requires highly motivated patients to ensure a satisfactory drug adherence.

Immune based therapies

Innumerable possibilities of immune based therapies can be considered. Only interleukin 2 (IL-2) has been intensively studied so far and used as an adjunctive therapy to standard antiretroviral drug regimens.[68–72] IL-2 was safe and induced a rapid recovery of CD4+ T cells. CD8+ T lymphocyte counts were not affected and plasma HIV RNA showed no significant changes. The rise of CD4+ T lymphocytes consisted of an elevation of naive and memory T cell subsets[68 72] and was dependent on

baseline levels of CD4+ T cells.[71] No restoration of disrupted TCR repertoires has been observed.[68] Delayed type hypersensitivity (DTH) responses improved significantly in one study,[69] but showed no significant changes in two other studies.[70 71] However, in one study an enhancement of proliferative lymphocyte responses to at least one tested mitogen or recall antigens has been observed in all 13 studied subjects on IL-2 therapy.[71]

The persistence of elevated CD4+ T cell numbers after discontinuation of IL-2 suggests that CD4+ T cell increases are most likely the result of a regeneration of new T cells and not simply of a redistribution of lymphocytes from lymphoid tissue.[69 71] IL-2 may therefore be a valuable addition to a standard antiretroviral therapy in order to increase the number and preserve the repertoire of CD4+ T lymphocytes. Moreover, T cell function may be enhanced in certain individuals.

Clinical impact of immune restoration in subjects on HAART

Improvements of immunological laboratory variables have been corroborated by the declining incidence of opportunistic infections such as cytomegalovirus (CMV) retinitis, *Pneumocystis carinii* pneumonia (PCP) and disseminated mycobacterial infections or by the reduction of the overall mortality from AIDS defining events.[73–76] Previously untreatable opportunistic infections such as cryptosporidiosis, microsporidiosis, azole resistant oropharyngeal candidiasis, or progressive multifocal leucoencephalopathy may resolve spontaneously after the initiation of HAART.[74 77–87]

A further clinical observation includes a high incidence of opportunistic infections during the first 2 months after initiation of HAART despite an increase of absolute CD4+ T cell counts.[88] Similarly, CMV retinitis was observed in individuals despite CD4+ T cells counts of more than 100 cells $\times 10^6$/l.[89 90] Localised *Mycobacterium avium* complex infections and an increasing number of subjects with herpes zoster infections have been reported after the initiation of HAART.[91 92] Those observations indicate that the enhancement of the immune response by HAART may unmask previously unrecognised subclinical opportunistic infections.

Recent reports of long term remission of CMV or *Mycobacterium avium* complex infections after discontinuation of the secondary prophylaxis provide some evidence that a degree of immune competence can be achieved which permits the discontinuation of the antimicrobial prophylactic therapy.[79 93–95] In a recently published study, primary prophylaxis against PCP pneumonia has been discontinued after CD4+ T lymphocyte counts increased to levels above 200 cells $\times 10^6$/l for at least 3 months.[11] During a median 11 months follow up period no new cases of PCP or toxoplasmic encephalitis have been noted. Similarly, primary and secondary PCP prophylaxis have been successfully discontinued in 62 and 16 subjects, respectively, after CD4+ T cell counts increased above 200 cells $\times 10^6$/l.[96] Those observations suggest that it is safe to discontinue prophylactic therapy after the number of CD4+ T lymphocytes increases above a critical threshold. The clinical impact of HAART is summarised in table 2.

Table 2 Clinical observation in subjects on HAART

Reduced HIV-1 associated mortality
Reduced incidence of AIDS defining events
Reduced incidence of opportunistic infections
Spontaneous resolution of opportunistic infections
Unmasking of subclinical opportunistic infections

Conclusion

In summary, recent studies suggest that not only do the number of CD4+ T cells improve, but also T lymphocyte function is at least partially restored by HAART within 1–2 years. The addition of immune based therapies to the standard antiretroviral drug regimen may shorten the time of the recovery of the immune system in subjects with advanced stages of the disease. Laboratory signs of immune reconstitution translated into clinical observations of a declining incidence of opportunistic infections and HIV-1 associated mortality. A limited number of small studies provide some evidence that primary and secondary prophylactic therapy can be safely discontinued when CD4+ T lymphocytes increase above a critical threshold. Preliminary data of immune reconstitution in subjects treated with HAART during acute HIV-1 infection are promising and indicate that an early initiation of therapy may facilitate the correction of HIV-1 associated immunodeficiency. A full restoration of the immune system has not been observed so far. However, it has to be considered that the observation period of 1–2 years is still a relatively short time. The long term follow up of individuals on HAART will show what level of immune reconstitution can be achieved.

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